Amendments to the Specification

At page 1, line 1, please insert the following new paragraph:

--The present application is a division of U.S. Application No. 09/445,283, filed December 3, 1999 which claims priority to International Application No. PCT/US98/11437, filed June 3, 1998, which claims priority to U.S. Application No. 60/058,670, filed September 12, 1997 and U.S. Application No. 60/048,376, filed June 3, 1997, the entire disclosures of each being incorporated by reference herein.--

At page 18, line 6-15, please replace the table with the following new table:

Gene	5' nt position in ptDNA	Sequence	SEQ ID NO:
clpP#2	70241	GGTCTAGACTACACTTTAATATGGA	(44)
clpP#3	70549(C)	GGGAATTCTGTTTGTAAGAAGA	(45)
atpB#2	56640	GGTCTAGAATTCCTATCGAATTCCTTC	~~(46)~~ (41)
atpB#3	56832(C)	GGCTCGAGGGACAACTCGATAGGATTAGG	~~(47)~~ (46)
rpoB#3	21394(C)	GGTCTAGAATCTAGCAATCATGGAATC	~~(48)~~ (47)
rpoB#4	21066	GGCTCGAGCGTGCTATTCTAAATCGT	~~(49)~~ (48)

At page 21, lines 18-32, please replace the paragraph with the following new paragraph:

Interestingly, each maize NEP promoter has sequence similarity around the transcription initiation site with the loose dicot NEP promoter consensus CATAGAATA/GAA (SEQ ID NO:63) (Hajdukiewicz et al., 1997, *supra*; underlined in Fig. 5). For clpP, 9 nucleotides are conserved out of 10; for atpB and rpoB, the number of conserved nucleotides is 7 out of 10 (Fig. 5). In addition, a second conserved region (Box II, Figure 5) is found upstream of Box I in atpB and clpP, but not in the rpoB promoters. Interestingly, the moncot Box II

contains truncated versions of the dicot NEP consensus in a direct orientation: 7 out of 10 bp match in case of the maize clpP (ATAGAAT) and atpB (AT-GAATA) genes (Fig. 5). These tandem repeats may play a role in regulating NEP promoter activity.

At page 22, line 4-page 23, line 3, please replace the paragraphs with the following new paragraphs:

The data reported herein show that maize plastid NEP promoter regions share sequence homology around the transcription initiation site with the conserved CATAGAATA/GAA (SEQ ID NO:63) NEP sequence motif in tobacco (Fig. 5). Therefore, these promoters are considered to be Type I NEP promoters. This finding indicates conservation of the NEP transcription machinery between monocots and dicots. Sequences upstream of the transcription initiation sites are conserved more extensively than downstream sequences among the maize clpP, rpoB, and atpB promoters, as shown in Figure 5, similar to the dicot Type I NEP promoters (Hajdukiewicz et al., 1997, *supra*).

Both, the maize PclpP-111 promoter and the tobacco PclpP-53 promoter are constitutive. In contrast to the maize promoter, the clpP promoter region in tobacco lacks the CATAGAATA/GAA (SEQ ID NO:63) sequence motif (Hajdukiewicz et al., 1997, *supra*) suggesting recognition by a different NEP specificity factor (Type II NEP promoter). Interestingly, in Type II NEP promoters sequences downstream of the transcription initiation sites are conserved more extensively than upstream as described in the following example. The tobacco PclpP-53 promoter homologues are the only known examples for plastid Type II NEP promoters. They have been highly conserved during evolution, including the liverworth Machantia polymorpha and the conifer Pinus contorta. Although DNA sequences required for clpP Type II NEP promoter

function are maintained, this region is transcriptionally silent in maize, rice and wheat. Lack of transcription from this region in cereals is probably due to the loss of Type II recognition specificity. See Example II. Accordingly, the tobacco (dicot) Type II clpP promoter is suitable to drive the expression of plastid transgenes only in dicots, whereas the cereal Type I promoter may be useful in both monocots and dicots.

At page 24, line 33- page 25, line 14, please replace the paragraph with the following new paragraph:

Plasmid pDS44 is a pLAA24 derivative (Zoubenko et al., *Nucleic Acids Res.* **22**:3819-3824, 1994) which carries a uidA reporter gene expressed from a Prrn promoter. Plasmid pDS44 was obtained by excising the Prrn promoter as an SacI/EcoRI fragment and replacing it with the rice clpP promoter region engineered as a SacI/EcoRI fragment. The 251 nucleotide SacI/EcoRI DNA fragment containing the rice clpP promoter region (including 19 basepairs of the coding region) was obtained by PCR amplification. The sequence of the PCR primers, and the position of their first nucleotide (or of its complement) in the rice plastid genome (Hiratsuka et al., *Mol. Gen. Genet.* **217**:185-194, 1989; GeneBank Accession No. X15901) are:

P1 68520(C) gggagcTCGAATCACCATTCTTT SEQ ID NO: ~~50~~ 49

P2 68270 gggaattcTTGGAACACCAATGGGCAT SEQ ID NO: ~~51~~ 50

Nucleotides derived from the plastid genome are in capital letters; those included to create a restriction site are in lower case letters. SacI or EcoRI restriction sites are underlined.

At page 25, line 33- page 26, line 2, please replace the paragraph with the following new paragraph:

Total leaf RNA was isolated from leaves of in vitro grown plants by the method of Stiekema et al., *supra*. Primer extension reactions were carried out on 20 μg of RNA with primer uidA PE1 as described by Allison and Maliga (1995) using primer P3: 5'-GGCCGTCGAGTTTTTTGATTTCACGGGTTGGGG-3' (SEQ ID NO:~~52~~ 51) (which is complementary to the uidA coding region.

At page 29, line 14- page 30, line 18, please replace the paragraphs with the following new paragraphs:

Construction of Plasmids. Plasmid pPS8 contains a uidA reporter gene as a SacI-HindIII fragment in a pBSKS+ plasmid (Stratagene). The chimeric uidA gene consists of: Between the SacI and XhoI sites, the PclpP-53(-22/+25) promoter fragment containing 22 nt upstream and 25 nt downstream (+1 is nt where transcription initiates) of the clpP transcription initiation site; Between XhoI and NcoI sites, a ribosome binding site; Between the NcoI and XbaI sites, the uidA coding region with an N-terminal c-myc tag corresponding to amino acids 410-419 (EQKLISEEDL; SEQ ID NO: ~~53~~ 52) within the carboxy terminal domain of the human c-myc protein (Kolodziej and Young, *Meth. Enz.* **194**:508-519, 1991); Between the XbaI and HindIII sites the 3' untranslated region of the rps16 ribosomal protein gene (Trps16). DNA sequence of the chimeric uidA gene between the SacI and HindIII sites in plasmid pPS8 is shown in Figure 13. Relevant restriction sites of the chimeric uidA gene are shown in Fig. 10, where the uidA gene is shown as part of plasmid pPS18. Plasmid pPS18 was obtained by cloning the uidA gene as a SacI-HindIII fragment into SacI-HindIII-digested pPRV111A plastid transformation vector. Plasmid pPRV111A, a pBSKS+ plasmid derivative (Strategene), and was described in Zoubenko et al., 1994, *supra*.

Plasmids pPS16, pPS37, pPS17 and pPS38 listed in Figure 11 were obtained from plasmid pPS18 by replacing the

PclpP-53(-22/+25) SacI-XhoI promoter fragment with the PclpP-53(-152/+154), PclpP-53(-152/+41), PclpP-53(-152/+10), PclpP-53(-39/+154) promoters, respectively. The SacI-XhoI fragments were obtained by PCR amplification. PCR primers are listed according to the position of the terminal nucleotide relative to the transcription initiation site (it is the complement of nt 74557 in the tobacco plastid genome: accession no. Z00044):

clpP-152	ccgagctcGAATGAGTCCATACTTAT	SEQ ID NO: ~~54~~ 53
clpP-39	ccgagctcAAAACCAATATGAATATTATA	SEQ ID NO: ~~55~~ 54
clpP -22	ccgagctcTATAAAGACAATAAAAAAAAT	SEQ ID NO: ~~56~~ 55
clpP+10	ccctcgaGAAACGTAACAATTTTTTTT	SEQ ID NO: ~~57~~ 56
clpP+25	ccctcgagTTTCACTTTGAGGTGGA	SEQ ID NO: ~~58~~ 57
clpP+41	ccctcgagAGAACTAAATACTATATTTC	SEQ ID NO: ~~59~~ 58
clpP+154	ccctcgagATATGACCCAATATATCTG	SEQ ID NO: ~~60~~ 59

At page 30, line 31-page 31, line 1, please replace the paragraph with the following new paragraph:

Primer Extension Analysis. Total leaf RNA was isolated from the leaves of transgenic plants maintained on RM medium, by the method of Stiekema et al.,1988, *supra*. Primer extension reactions were carried out as described by Allison and Maliga, 1995, *supra*, using 15 μg of the total RNA and primer PE1 complimentary to the 5' end of the uidA coding sequence.

Primer PE1 sequence: 5'-GGCCGTCGAGTTTTTTGATTTCACGGGTTGGGG-3' (SEQ ID NO: ~~61~~ 51)

At page 32, lines 13-28, please replace the paragraph with the following new paragraph:

Sequences required for transcription by the NEP polymerase are not known. Based on conservation of the ATAGAATA/GAA (SEQ ID NO:64) around the transcription initiation site, Hajdukiewicz et al., 1997, *supra*, most NEP promoters were classified as Type I. The promoter studied

here, PclpP-53, lacks this sequence motif, because of which it is classified as Type II. Transcription analysis in the truncated promoter fragment of plasmid pPS18 in vivo shows that sequences required to support transcription from PclpP-53 are located within a 30 basepair fragment extending from -5 to +25 with respect to the transcription initiation site. Furthermore, nucleotides between +10 and +25 are important for transcription initiation, since there is no transcription from clpP promoter derivatives in plasmid pPS17 and pPS41 lacking this region.

At page 35, lines 9-16, please replace the table with the following new table:

Gene	5' nt position in plastid DNA	Sequence	SEQ ID NO:
atpB (Z.m.)	55860(C)	GAGAGGAATGGAAGTGATTGACA	~~(62)~~ (33)
	55103	GAGCAGGGTCGGTCAAATC	~~(63)~~ (34)
clpP (Z.m.)	69840	ATCCTAGCGTGAGGGAATGCTA	~~(64)~~ (35)
	70064(C)	AGGTCTGATGGTATATCTCAGTAT	~~(65)~~ (36)

At page 35, lines 39-45, please replace the table with the following new table:

Gene	5' nt position in plastid DNA	Sequence	SEQ ID NO:
rbcL	54124 (C)	ACTTGCTTTAGTTTCTGTTTGTGGTGACAT	~~(66)~~ (60)
atpB	53287	AGAAGTAGTAGGATTGGTTCTCATAAT	~~(67)~~ (61)
16S rRNA	123777	CCGCCAGCGTTCATCCTGAGC	~~(68)~~ (62)
clpP	68263	GGTACTTTTGGAACACCAATGGGCAT	~~(69)~~ (39)

Please replace the sequence listing from pages 39, line 9- page 54, line 48 with the paper copy of the sequence listing appended hereto.